

1. AMENDMENTS

Please cancel claims 1 to 38 without prejudice.

Please add the following new claims.

--39. A method for producing a library of expressible open reading frames, the method comprising:

a) amplifying deoxyribonucleic acid (DNA) molecules comprising a plurality of open reading frames (ORFs) using a primer pair, wherein the primer pair comprises a 5' primer, which comprises a nucleotide sequences starting 5'-CACCATG, thereby producing a plurality of amplified ORFs;

b) inserting amplified ORFs of the plurality into an expression vector, thereby producing expression vectors comprising the amplified ORFs; and

c) verifying the size and orientation of the amplified ORFs in the expression vectors, thereby producing a library of expressible ORFs.

40. The method of claim 39, wherein the primer pair further comprises a 3' primer, which causes the amplification product to end at the third position of a codon immediately preceding a stop codon of an ORF being amplified.

41. The method of claim 40, wherein the 3' primer further causes the amplification product to comprise a 3' terminal adenine residue.

42. The method of claim 39, further comprising transforming cells with the expression vectors comprising the amplified ORFs, thereby obtaining a library of transformed cells containing the expression vectors.

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43. The method of claim 39, further comprising purifying the amplified ORFs prior to inserting the amplified ORFs into the expression vector.

44. The method of claim 43, wherein purifying the amplified ORFs is performed using column chromatography or gel electrophoresis.

45. The method of claim 43, wherein purifying the amplified ORFs is performed using agarose gel electrophoresis.

46. The method of claim 45, wherein the agarose is low melt agarose.

47. The method of claim 39, wherein amplified ORFs of the plurality encode full length proteins.

48. The method of claim 39, wherein inserting the amplified ORFs into the expression vector is performed using an enzyme that cleaves and ligates DNA.

49. The method of claim 48, wherein the enzyme is a type I topoisomerase or a site-specific recombinase.

50. The method of claim 48, wherein the enzyme is a vaccinia DNA topoisomerase, a lambda integrase, an FLP recombinase, or a P1-Cre protein.

51. The method of claim 48, wherein the enzyme is a vaccinia DNA topoisomerase.

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52. The method of claim 39, wherein the expression vector is a eukaryotic expression vector.

53. The method of claim 39, wherein the expression vector is a prokaryotic expression vector.

54. The method of claim 39, wherein the expression vector is suitable for prokaryotic expression and eukaryotic expression.

55. The method of claim 39, wherein verifying the size and orientation of the ORF is performed using a polymerase chain reaction protocol.

56. The method of claim 40, wherein verifying the size and orientation of the ORF is performed using whole cell lysates of transformed cells containing the expression vectors.

57. The method of claim 39, wherein the DNA molecules comprise prokaryotic DNA or eukaryotic DNA.

58. The method of claim 57, wherein the eukaryotic DNA is obtained from yeast cells or mammalian cells.

59. The method of claim 39, wherein the amplified ORFs of the plurality encode members of a family of proteins.

60. The method of claim 59, wherein the members of the family of proteins are human proteins.

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61. The method of claim 59, wherein the members of the family of proteins comprises members of a family of kinases, phosphatases, transcription factors, oncogenes, or tumor suppressors.

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62. The method of claim 39, which is performed in a high throughput format.

63. The method of claim 39, which is performed in a multiwell microtiter plate.

64. The method of claim 40, further comprising the additional step of verifying the size and orientation of the ORF in the expression vector in the transformed cells.

65. The method of claim 40, wherein the transformed cells are eukaryotic cells or prokaryotic cells.

66. The method of claim 40, wherein the transformed cells are bacteria, yeast, fungi, insect cells, mammalian cells, or plant cells.

67. The method of claim 40, wherein the transformed cells Chinese hamster ovary cells or *Saccharomyces cerevisiae* cells.

68. The method of claim 39, wherein the expression vector comprises a nucleotide sequence encoding an affinity purification tag or an epitope tag, and wherein the expressible ORF and the nucleotide sequence encode a fusion protein comprising a polypeptide encoded by the ORF and the tag.

69. A library of expressible ORFs produced according to the method of claim 39.

70. The library of claim 69, wherein the ORFs encode yeast proteins or human proteins.

71. A method for producing a library of selected expressible open reading frames (ORFs), the method comprising:

- a) amplifying deoxyribonucleic acid (DNA) molecules comprising a plurality of ORFs using a primer pair, wherein the primer pair comprises a 5' primer, which comprises a nucleotide sequences starting 5'-CACCATG, and a 3' primer, which causes the amplification product to end just prior to a stop codon, thereby producing a plurality of amplified ORFs;
- b) purifying amplified ORFs of the plurality, thereby obtaining purified amplified ORFs;
- c) inserting the purified amplified ORFs into expression vectors using a vaccinia DNA topoisomerase, thereby producing expression vectors comprising the amplified ORFs;
- d) transforming cells with the expression vectors comprising the amplified ORFs; and
- e) selecting transformed cells containing expression vectors comprising ORFs in an orientation for expression of a polypeptide encoded by the ORF.

72. The method of claim 71, wherein purifying the amplified ORFs comprises separating the amplified ORFs using agarose gel electrophoresis, and isolating the amplified ORFs from the agarose gel.

73. The method of claim 72, wherein the agarose is low melt agarose.

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74. The method of claim 71, wherein inserting the purified amplified ORFs into the expression vectors is performed using an enzyme that cleaves and ligates DNA.

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75. The method of claim 74, wherein the enzyme is a vaccinia DNA topoisomerase, a lambda integrase, an FLP recombinase, or a P1-Cre protein.

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76. The method of claim 74, wherein the expression vectors are suitable for prokaryotic expression and eukaryotic expression.--
